Characterization of a Segregation Distortion Locus with Powdery Mildew Resistance in a Wheat-*Thinopyrum intermedium* Introgression Line WE99

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Abstract

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Exploitation of host resistance is important for controlling powdery mildew of wheat (*Triticum aestivum* L.). In this study, a wheat-*Thinopyrum intermedium* introgression line, designated WE99, conferred seedling resistance to 47 of 49 *Blumeria graminis* f. sp. *tritici* isolates. Genetic analysis demonstrated that the resistance segregation deviated significantly from a single gene Mendelian ratio. However, marker analysis indicated that only a single recessive resistance gene, temporarily designated *PmWE99*, conferred powdery mildew resistance (*Pm*). *PmWE99* was mapped to chromosome arm 2BS and linked to the three simple-sequence repeat markers *Gwm148*, *Gwm271*, and *Barc55*. Using race

spectrum analysis, *PmWE99* was shown to be significantly different from the documented genes *Pm42* and *MIW170* located on chromosome arm 2BS and, thus, appeared to be a new *Pm* gene. Examination of the genotype frequencies in the $F_{2,3}$ families showed that a genetic variation in the *PmWE99* interval that favored the transmission of the WE99 allele could be the cause of the deviated segregation. Further investigation revealed that the abnormal segregation only occurred at the *PmWE99* interval and was not common at other loci in this population. Identification of *PmWE99* will increase the diversity of the *Pm* genes for wheat improvement.

Wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a devastating disease, which can cause severe yield losses in a relatively short time (Morgounov et al. 2012). Chemical protection, specific agronomic management practices, and the breeding of resistant cultivars were several solutions that had been used to prevent epidemics of this disease (Hardwick et al. 1994; Horst 2013; Sharma et al. 2004). Undoubtedly, host resistance is the most effective, economical, and environmentally sound and consistently used method (Petersen et al. 2014).

Up to now, more than 70 formally designated powdery mildew resistance (*Pm*) genes or alleles (*Pm1* to *Pm54*) and 20 temporarily designated *Pm* genes have been identified at 49 loci distributed on all the chromosomes except chromosome 3D in common wheat and its relatives (Ma et al. 2015a,b; McIntosh et al. 2014). An increasing number of studies showed the diversity in powdery mildew resistance. Most *Pm* genes displayed dominant inheritance; however, a dozen *Pm* genes confer recessive resistance, such as the *Pm5* alleles (*Pm5a* to *Pm5e*) (Hsam et al. 2001; Huang et al. 2003), *Pm9* (Schneider et al. 1991), *Pm26* (Rong et al. 2000), *Pm42* (Hua et al. 2009), and several temporarily designated *Pm* genes (Sun et al. 2006; Xu et al. 2008). Qualitative resistance is common in documented *Pm* genes; in contrast, several of them are quantitatively inherited, such as *Pm38* (Spielmeier et al. 2008), *Pm39* (Lillemo et al. 2008), *Pm46* (Herrera-Foessel et al. 2014), *Pm54* (Hao et al. 2015), and others. Most qualitative resistance for powdery mildew followed Mendel's law of segregation; however, a few segregated abnormally, such as *PmX* (Fu et al. 2013) and two stem rust resistance genes, *Sr36* (Tsilo et al. 2008) and *Sr40* (Wu et al. 2009).

Although numerous *Pm* genes have been identified, most of them are race specific and liable to the breakdown of resistance once they become widely deployed in wheat cultivars (Chen 2005; Xiao et al. 2013). Identification and utilization of new *Pm* genes from various wheat germplasm resources is a continuous challenge. In the past, the discovery of novel resistance sources mainly focused on cultivated wheat (McIntosh et al. 2014). However, high genetic homogeneity within the cultivated species creates unprecedented challenges to wheat breeding (Gupta et al. 2010; Karsai et al. 2012; Khan et al. 2011). Transfer of resistance genes from wheat relatives is an important strategy for resistance breeding. In fact, wheat relatives have been used as genetic resources for the introgression of powdery mildew resistance into the wheat genome, such as the genes *Pm40* and *Pm43* from *Thinopyrum intermedium* (He et al. 2009; Luo et al. 2009); *Pm21* from *Haynaldia villosa* (Chen et al. 1995); *Pm7*, *Pm8*, *Pm17*, and *Pm20* from *Secale cereale* (Friebe et al. 1994, 1996; Heun et al. 1990; Hsam and Zeller 1997), *Pm26* and *Pm42* from *Triticum turgidum* var. *dicoccoides* (Hua et al. 2009; Rong et al. 2000); and *MI5253* from *T. turgidum* subsp. *dicoccum* (Piarulli et al. 2012).

In this study, a wheat distant hybridization descendant crossed by common wheat and *Thinopyrum intermedium*, designated WE99, showed powdery mildew resistance at both seedling and adult stages. The objectives of the present study were to (i) detect *T. intermedium* chromatin in WE99 and confirm its chromosome composition, (ii) analyze the inheritance of its seedling resistance to powdery mildew, (iii) determine the chromosomal location of the resistance genes using molecular markers, and (iv) compare its reaction pattern with other documented *Pm* genes using resistance spectrum analysis.

Materials and Methods

Plant materials. Wheat line WE99 was derived from a cross between common wheat and *T. intermedium* (the pedigree is shown in Supplementary Figure S1). 'Huixianhong' wheat served as susceptible parent in a cross with WE99 to produce F_1 hybrids, F_2 populations, and $F_{2,3}$ families for genetic analysis and molecular mapping of resistance genes. Common wheat lines 2H7165 (with *Pm42*) and P170 (with *MIW170*) were derived from the documented *Pm42* and *MIW170* donor wild emmer accessions G-303-1M and

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P170, respectively, and kindly provided by Professor Zhiyong Liu (China Agricultural University, Beijing). They were used in the comparisons of their *Pm* genes with that of WE99. Huixianhong was used as the susceptible check.

Molecular cytological detection of *T. intermedium* chromatin in WE99. Genomic in situ hybridization (GISH) analysis was conducted for detection of *T. intermedium* chromatin in WE99 and to determine its chromosomal composition. To confirm the genetic stability, WE99 was examined during five consecutive wheat growing seasons. Each year, five plants were examined and five root tip cells were analyzed per plant. The mitotic chromosomes of root tips cells of WE99 were prepared and observed following the method of Han et al. (2006). Total genomic DNA of *T. intermedium* was extracted (Sharp et al. 1988) and used as a probe after labeling with fluorescein-12-dUTP by the nick translation method (Han et al. 2007). Detection and visualization of the chromosomes were performed as described by Han et al. (2009). The cells were captured by a DVC CCD digital camera, and program Image Pro Plus 4.0 was used for merging each color channel from the captured images.

In order to identify the *T. intermedium* chromatin in WE99 and its relationship to the powdery mildew resistance genes, 20 simple-sequence repeat (SSR) markers that produce specific bands from the *T. intermedium* genome (Liu et al. 2013) were used to amplify WE99, *T. intermedium*, Huixianhong, 'Chinese Spring', and several homozygous resistant and susceptible $F_{2:3}$ families from the cross of Huixianhong to WE99.

Disease assessment. At the adult stage, WE99 was evaluated for its adult resistance to a mixture of *Bgt* isolates collected from northern China in five consecutive wheat growing seasons. At the one-leaf stage, seedlings were inoculated with fresh spores using the dusting method (An et al. 2013). They were then transferred to a greenhouse where the environment was maintained at high humidity and 18°C (day) to 12°C (night), with a photoperiod of 12 to 14 h of light per day. Infection types (IT) were scored 14 to 15 days after inoculation when pustules were fully developed on susceptible controls 'Mingxian 169' and Huixianhong. The IT of each plant was recorded on a 0-to-4 scale, in which the plants with IT 0 to 2 were classified as a resistant and IT 3 to 4 as susceptible phenotypes for powdery mildew (Si et al. 1992).

Line WE99 was tested for its reactions to 49 single-pustule-derived *Bgt* isolates by separate artificial inoculation in a temperature-controlled greenhouse (Supplementary Table S1). Wheat lines 2H7165 and P170, several resistant commercial cultivars that are currently popular in China (i.e., 'Jimai 22', 'Liangxing 66', 'Liangxing 99', and 'Wennon 14'), the susceptible parent Huixianhong, and the susceptible control Mingxian 169 were also included in the tests. Each isolate was placed in an independent and enclosed space to avoid cross-infection between different isolates. Each test was repeated three times, and five plants were included in each test. Meanwhile, 40 plants of each $F_{2:3}$ family of Huixianhong and WE99 were inoculated

with isolate B03 and the IT of each plant was scored for genetic and molecular marker analysis.

Molecular marker analysis. Equal amounts of genomic DNA from 10 resistant (with IT 0) and 10 susceptible (with IT 4) F_2 plants were extracted from fresh leaves after disease assessment of the F_2 population (Sharp et al. 1988). They were bulked to produce resistant and susceptible pools for bulked segregant analysis (BSA). In total, about 400 SSR markers distributed on all 21 wheat chromosomes were tested for polymorphisms between the parents and bulks. Then, F_2 population were transplanted in the field to harvest $F_{2:3}$ families. Genomic DNA of each $F_{2:3}$ family was extracted from fresh leaves after disease assessment (Sharp et al. 1988). Markers showing polymorphisms between the parents and bulks were used to genotype all 184 $F_{2:3}$ families. The procedures for polymerase chain reaction (PCR), separation, and visualization of the PCR products followed the method of Xu et al. (2012), and the PCR profile was modified as follows: 1 cycle at 94°C for 3 min; followed by 35 cycles at 94°C for 30 s, 50 to 60°C (depending on the specific primer) for 40 s, and 72°C for 40 s; and a final extension at 72°C for 5 min.

Map construction. Goodness-of-fit of segregation data with hypothesized 3:1 and 1:2:1 ratios for F_2 population and $F_{2:3}$ families were evaluated with χ^2 tests using Sigmaplot 2001 software (SPSS Inc., Chicago). Linkage maps with genetic distances between markers and the resistance gene were prepared using Software MAPMAKER 3.0 with a log of the likelihood threshold of 3.0 (Lincoln et al. 1992) using a total of 184 $F_{2:3}$ families from the cross of Huixianhong to WE99. Recombination fractions were converted to map distances (in centimorgans) using the Kosambi mapping function (Kosambi 1943).

Results

Detection of the *T. intermedium* chromatin in WE99. Wheat line WE99 was selected from distant hybridization progenies from a cross of common wheat and *T. intermedium*. GISH analysis using *T. intermedium* genomic DNA as a probe was first used to detect *T. intermedium* chromatin and determine the chromosome constitution of WE99. The result revealed that WE99 had 42 chromosomes at the mitotic metaphase as common wheat (Fig. 1). These chromosomes showed only blue signals when counterstained with 4,6-diamidino-2-phenylindole and no bright-green hybridization signals were detected, indicating that there were cytologically undetectable *T. intermedium* chromatin segments on the wheat chromosomes. Therefore, no *T. intermedium* chromosome segments were detected cytologically in WE99.

In order to further confirm these results, 20 molecular markers that produce specific bands from the *T. intermedium* genome (Liu et al. 2013) were used to detect *T. intermedium* chromatin in WE99. The results demonstrated that the SSR marker *Wmc382* located on chromosome arm 2BS amplified a 175-bp band that was specific for *T. intermedium* chromatin in WE99 (Fig. 2). This indicated that,

Fig. 1. Genomic in situ hybridization (GISH) detection of the wheat-*Thinopyrum intermedium* introgression line WE99 using *T. intermedium* genomic DNA as a probe **A**, clearly showed 42 wheat chromosomes counterstained with 4,6-diamidino-2-phenylindole (DAPI) and no bright green hybridization signals representing *T. intermedium* chromatin. **B**, GISH pattern of a small alien segment translocation with detectable bright green hybridization signals was a positive control. Arrows indicate alien chromatin.

although *T. intermedium* chromatin was cytologically undetectable, WE99 indeed has a small *T. intermedium* introgression which may have high homology with some regions of chromosome arm 2BS (Fig. 2). Therefore, WE99 was proved to be a wheat-*T. intermedium* introgression line. Different progenies in five consecutive generations of selfing were also investigated by molecular cytological and agronomical observation. WE99 was genetically and agronomically stable, demonstrating that WE99 was a stable wheat-*T. intermedium* introgression line.

To investigate the relationship between the powdery mildew resistance in WE99 and the *T. intermedium* chromatin detected by the marker *Wmc382*, several homozygous resistant and susceptible $F_{2:3}$ families crossed by Huixianhong and WE99 were screened with the molecular marker *Wmc382*. The result indicated that not all of the resistant $F_{2:3}$ families had a *T. intermedium* segment and it was present in some of the susceptible $F_{2:3}$ families (Fig. 2). Therefore, the powdery mildew resistance was not contributed by the *T. intermedium* chromatin detected by the marker *Wmc382*.

Evaluation of powdery mildew resistance and its inheritance in WE99. At the adult plant stage, WE99 was highly resistant to the mixture of *Bgt* isolates collected from northern China in five consecutive wheat growing seasons. At the seedling stage, WE99 showed resistance to 47 of 49 tested *Bgt* isolates collected from different regions of China (Fig. 3). In contrast, several resistant commercial cultivars that are currently popular in China, which

included Jimai 22, Liangxing 66, Liangxing 99, and Wennong 14, each had a relatively narrower resistant spectrum. Therefore, WE99 had more effective resistance to wheat powdery mildew compared with several resistant commercial cultivars that are widely grown in China.

To investigate the inheritance of the seedling resistance to powdery mildew, *Bgt* isolate B03, which is the dominant race in North China, was selected to inoculate WE99, Huixianhong, and their derived $F_{2:3}$ families. The results demonstrated that WE99 was highly resistant, with an IT 0, while the susceptible parent Huixianhong was highly susceptible, with an IT 4. The F_1 hybrids from the cross of Huixianhong to WE99 were susceptible, with IT 3 to 4, indicating the recessive characteristics of the powdery mildew resistance in WE99. The F_2 populations of Huixianhong $\times$ WE99 segregated 75 resistant to 120 susceptible plants. The resistance segregation did not produce a good fit of the Mendelian ratio for one recessive resistance gene ($\chi^2_{1:3} = 18.85$, $df = 1$, $P = 1.40$) but it seemed to fit the Mendelian ratio of two recessive resistance genes ($\chi^2_{7:9} = 2.22$, $df = 1$, $P = 0.14$). Then, F_2 populations were transplanted to the field, and 184 plants survived to produce F_3 seed. In all, 40 plants of each $F_{2:3}$ family were tested for their powdery mildew response to the *Bgt* isolate B03, and they segregated as 72 homozygous resistant, 97 segregating, and 15 homozygous susceptible, also fitting Mendelian ratio for two recessive resistance genes ($\chi^2_{7:8:1} = 2.23$, $df = 2$, $P = 0.33$).

Fig. 2. Molecular detection of the wheat-*Thinopyrum intermedium* introgression line WE99 using a simple-sequence repeat marker specific for *T. intermedium* genome and amplification of several resistant and susceptible F_2 plants crossed by Huixianhong and WE99. Lane M, pUC18 *Msp*I; lane 1, Chinese Spring; lane 2, *T. intermedium*; lane 3, WE99; lane 4, Huixianhong; R = resistant and S = susceptible F_2 plants.

Fig. 3. Reactions of WE99 and the wheat lines 2H7165 with *Pm42* and *P170* with *MIW170* to several selected *Blumeria graminis* f. sp. *tritici* (*Bgt*) isolates selected from the 49 *Bgt* isolates in this study. Mingxian 169 wheat was used as the susceptible control.

When two independent recessive genes segregate in a Mendelian ratio, about half of the $F_{2:3}$ families with phenotype segregation would have one-quarter of plants with the recessive phenotype, and the other half would follow the segregation ratio of two recessive resistance genes that fit a ratio of 7:9 resistant/susceptible plants. However, the results indicated that, within each of the 97 $F_{2:3}$ families with segregating phenotypes, there were no $F_{2:3}$ families in which one-quarter of plants had a recessive phenotype, and nearly all of the 97 $F_{2:3}$ families with phenotype segregation followed the segregation ratio of two recessive resistance genes. Therefore, it was speculated that abnormal segregation might exist within the population and the conclusion of two independent recessive genes segregation might not be correct.

Molecular mapping of the powdery mildew resistance gene in WE99. After screening 400 SSR markers distributed on all 21 wheat chromosomes, 12 markers (*Wmc17*, *Wmc182*, *Wmc257*, *Wmc317*, *Wmc382*, *Barc55*, *Barc121*, *Gwm410*, *Gwm148*, *Gwm271*, *Gwm501*, and *Cfd70*) showed polymorphism between two parents and the resistant and susceptible bulks (Fig. 4). These resulting markers were then used to genotype the 184 $F_{2:3}$ families of Huixianhong and WE99. Only three markers (*Gwm148*, *Gwm271*, and *Barc55*) distributed on chromosome arm 2BS were linked to the powdery mildew resistance in WE99 at genetic distances of 10.4, 11.1, and 13.5 centimorgans (cM), respectively (Fig. 5). Thus, it was concluded that a single recessive gene on chromosome arm 2BS contributed to the powdery mildew resistance in WE99. This gene was temporarily designated *PmWE99*. The loci order of *PmWE99* and the linked markers was *PmWE99*–*Xgwm148*–*Xgwm271*–*Xbarc55* (Fig. 5).

Comparison of *PmWE99* and documented *Pm* genes on chromosome arm 2BS. In order to compare *PmWE99* with the documented *Pm* genes on chromosome arm 2BS, 49 *B. graminis* f. sp. *tritici* isolates were used to test the reaction patterns of WE99 and two resistance stocks 2H7165 and P170 with the documented *Pm* genes *Pm42* and *MIIW170*, respectively, on chromosome arm 2BS. The results demonstrated that WE99 was resistant to 47 of 49 isolates

tested, with an avirulence frequency of 95.9%, whereas 2H7165 and P170 only showed resistance to 38 and 33 of the 49 isolates tested, with lower avirulence frequencies of only 77.6 and 67.3%, respectively (Fig. 3). Therefore, WE99 has a significantly different resistant spectrum from the documented resistance stocks 2H7165 and P170 with *Pm42* and *MIIW170*, respectively.

Segregation distortion detection of *PmWE99*. From the mapping result, it appears that there was a single recessive gene in WE99; however, the segregation ratio of the resistant phenotypes in the F_2 and their derived $F_{2:3}$ families showed abnormal segregation. To clarify this issue, a number of plants corresponding to each genotype at *PmWE99* and the linked marker loci in the $F_{2:3}$ families were examined. The results showed that the genotype proportions of all the loci fit neither a 3:1 ratio for the single dominant markers *Gwm148* and *Barc55* nor a 1:2:1 ratio for codominant marker *Gwm271* (Table 1), which segregated abnormally. These results were consistent with the occurrence of segregation distortion of the phenotypes in the F_2 population and the $F_{2:3}$ families.

Further investigation revealed that marker loci that were closer to the centromere of chromosome 2BS showed more segregation distortion, including the locus of *PmWE99*; and, meanwhile, more genotypes of WE99 appeared closer to the centromere on chromosome 2BS (Table 1; Fig. 5). This result indicated that there may be a genetic variation in the interval between loci *PmWE99* and *Xbarc55* close to the centromere of chromosome 2BS in WE99, which strongly favored the transmission of gametes with the WE99 allele and, thus, caused abnormal segregation of *PmWE99* and its linked markers.

To clarify whether this abnormal segregation also existed at other loci in the $F_{2:3}$ families of WE99 and Huixianhong, some other marker loci (i.e., *Xwmc17*, *Xwmc182*, *Xwmc317*, *Xbarc121-1*, *Xbarc121-2*, *Xcfd70*, and *Xgwm410*) showing polymorphisms between two parents but not linked to *PmWE99* were examined for their segregation patterns (Table 1). Unlike *PmWE99* and its linked markers, the genotypic proportions at these loci in this population

Fig. 4. Examples of amplification patterns of *PmWE99*-linked markers **A**, *Gwm148*; **B**, *Barc55*; and **C**, *Gwm271*, which were detected in 8% nondenatured polyacrylamide gels. Arrows indicate the polymorphic bands. Lane 1, WE99; lane 2, Huixianhong; lane 3, resistant bulked pool; lane 4, susceptible bulked pool; lanes 5 to 14 (A and B), homozygous resistant families; lanes 15 to 24 (A and B), segregating or homozygous susceptible families; lanes 5 to 9 (C), homozygous resistant families; lanes 10 to 19 (C), segregating families; and lanes 20 to 24 (C), homozygous susceptible families.

all fit a 1:2:1 ratio perfectly. Therefore, the abnormal segregation only existed at the *PmWE99* interval and was not common for all the loci in this population.

Discussion

In this study, the wheat line WE99 crossed by common wheat and *T. intermedium* showed a high level of resistance to powdery mildew. Compared with several popular resistant commercial cultivars currently in China, it showed a broader resistance spectrum against 49 *B. graminis* f. sp. *tritici* isolates collected from different regions of China, indicating that it was a valuable resistance resource for wheat improvement. Using molecular marker detection, WE99 was proven to be a wheat-*T. intermedium* introgression line.

Using BSA and molecular markers, a recessive *Pm* gene, designated *PmWE99*, was detected on chromosome arm 2BS in WE99. Four documented *Pm* genes on chromosome arm 2BS have been identified, including *Pm26* that cosegregated with the restricted fragment length polymorphisms (RFLP) marker *Wg516* (Rong et al. 2000), *Pm42* that cosegregated with RFLP-derived sequence tagged site (STS) marker *BF146221* (Hua et al. 2009), *MIW170* that cosegregated with the RFLP-derived STS marker *Cau516* (Liu et al. 2012), and *MI5323* flanked by molecular markers *Cau516* and *CA695634* (Piarulli et al. 2012). These documented *Pm* genes all followed

standard Mendelian inheritance whereas, in contrast, *PmWE99* segregated abnormally. *PmWE99* was located on the wheat chromosome bin 2BS-0.53-0.75, based on previously reported maps (Hua et al. 2009; Somers et al. 2004), while resistance genes *Pm26*, *MIW170*, and *MI5323* were all located on 2BS-0.84-1.00. Both *MIW170* and *MI5323* were dominant genes, yet *PmWE99* was recessive. Therefore, *PmWE99* is different from *Pm26*, *MIW170*, and *MI5323* according to their genetic model, physical locations, and genetic characteristics. Interestingly, *Pm42* was only 3.7 cM from *PmWE99*, and they are both recessive genes (Hua et al. 2009; Somers et al. 2004). To distinguish *PmWE99* and *Pm42*, 49 *Bgt* isolates were used to test their reaction patterns to powdery mildew. The results indicated that *PmWE99* showed a significantly broader resistance spectrum than *Pm42*. Therefore, *PmWE99* also was different from *Pm42*, and appears to be a new *Pm* gene on chromosome arm 2BS. The allelic relationship between *PmWE99* and *Pm42* still needs to be determined in the future.

Segregation distortion is a phenomenon in plants, and the reasons are diverse. In wheat, inversion, alien translocation, and gametocidal genes could be several of the causes for the deviated segregation. For example, there was evidence that the homeologous group 2 chromosomes of *Triticeae* spp. carry genes favoring the transmission of specific gametes (Tsujimoto and Tsunewaki 1988). Fu et al. (2013)

Fig. 5. Linkage map of *PmWE99* and its loci comparison with the known *Pm* genes on chromosome arm 2BS using the anchoring marker loci of *Xgwm148* and *Xcau516*. Genetic distances are shown on the left in centimorgans. The black arrow points to the centromere.

Table 1. Plant numbers for each genotype at *PmWE99*, its linked marker loci, and some unlinked marker loci with polymorphisms between WE99 and Huixianhong in the $F_{2,3}$ families crossed by Huixianhong and WE99 and the χ^2 test against the expected Mendelian ratios

Marker loci	Number of each genotype ^a				Expected ratio	χ^2 Value ^b	Relationship ^c
	AA	AB	BB	A_B_			
<i>PmWE99</i>	69	91	14	...	1:2:1	81.78	Linked
<i>Xgwm148</i>	80	...	...	94	1:3	41.48	Linked
<i>Xgwm271</i>	85	78	11	...	1:2:1	64.01	Linked
<i>Xbarc55</i>	89	...	...	85	1:3	66.11	Linked
<i>Xwmc17</i>	58	41	83	...	1:2:1	4.58	Unlinked
<i>Xwmc182</i>	45	50	83	...	1:2:1	1.09	Unlinked
<i>Xwmc317</i>	57	40	86	...	1:2:1	3.82	Unlinked
<i>Xbarc121-1</i>	50	49	83	...	1:2:1	1.42	Unlinked
<i>Xbarc121-2</i>	40	47	95	...	1:2:1	0.89	Unlinked
<i>Xcfd70</i>	43	44	83	...	1:2:1	0.10	Unlinked
<i>Xgwm410</i>	47	47	79	...	1:2:1	1.30	Unlinked

^a A = WE99 allele, B = Huixianhong allele, A_ = the WE99 allele is dominant and the number includes both the WE99 genotype and the heterozygous genotype, and B_ = the Huixianhong allele is dominant and the number includes both the Huixianhong genotype and the heterozygous genotype.

^b Values; $\chi^2_{0.05, 1} = 3.84$ and $\chi^2_{0.05, 2} = 5.99$; $P = 0.01$.

^c Linkage relationship with *PmWE99*.

reported a recessive *Pm* gene (*pmX*) located on chromosome arm 2AL that showed abnormal segregation. The speculation was that there was a genetic variation linked to *pmX* favoring the transmission of gametes carrying it, similar to *PmWE99* in this study. Inheritances of the two stripe rust resistance genes *Sr36* and *Sr40* showed segregation distortion and recombination suppression which involved alien translocations (Tsilo et al. 2008; Wu et al. 2009). In this study, molecular genetic analysis of F_2 populations and their derived $F_{2:3}$ families from the cross of Huixianhong $\times$ WE99 demonstrated that WE99 carries a recessive gene for powdery mildew resistance. However, phenotype segregation of both F_2 populations and their derived $F_{2:3}$ families deviated significantly from single recessive gene inheritance. Further investigation of the variation pattern for the number of plants with the WE99 genotype at the loci along the *PmWE99* linkage map demonstrated that genetic variation in the *PmWE99* linkage map interval that favored the transmission of gametes carrying *PmWE99* may have resulted in a deviated segregation ratio. Further studies should be done to make clear the *PmWE99*-linked genetic variation that caused the segregation distortion. One case to note was that the *T. intermedium* chromatin detected by *Wmc382* was also introgressed into chromosome arm 2BS. This may not be a coincidence, implying the possibility that this *T. intermedium* introgression may result in a deviated segregation of powdery mildew resistance in WE99.

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